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71 Applicant:
Boehringer Mannheim GmbH, 6800 Mannheim, DE

72 Inventor:
Braun, Hans-Peter, Dr. rer. nat., 6944Hemsbach, DE;
Carstensen, Carsten Andreas, 6711 Heuchelheim, DE;
Deneke, Ulfert, Dr. rer. nat., 6942 Mörlenbach, DE;
Rothe, Anselm, Dr. rer .nat., 6943 Birkenau, DE

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54 **Rapid diagnostic test device and method for its use, in particular for quantitative determinations**

Rapid diagnostic test device comprising a substrate layer partially covered by an absorbent material, which in turn is partially covered by a reagent layer. The reagent layer is covered by a transparent surface layer and is affixed to the substrate layer and/or the absorbent material by means of a narrow zone in such a way that liquid contact between the reagent layer and the absorbent material does not occur until the surface layer is subjected to pressure; and method of using the rapid diagnostic test device, the sample being placed on the uncovered portion of the absorbent material, followed by distribution and temperature equilibration, after which the reagent layer is pressed flat and the reaction thus initiated is optically evaluated by reflective photometric methods.

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Claims

1. Rapid diagnostic test device comprising a substrate layer partially covered by an absorbent material, which in turn is partially covered by a reagent layer, characterized in that the reagent layer is covered by a transparent surface layer and is affixed to the substrate layer and/or the absorbent material by means of a narrow zone in such a way that liquid contact between the reagent layer and the absorbent material does not occur until the surface layer is subjected to pressure.

2. Rapid diagnostic test device according to Claim 1, characterized in that the portion not covered by the reagent layer is covered with an additional filter or separating layer.

3. Rapid diagnostic test device according to Claim 1 or 2, characterized in that the absorbent material and the reagent layer are in contact with one another via a hydrophobic grid.

4. Method of using the rapid diagnostic test device according to Claims 1 through 3, characterized in that the sample is placed on the uncovered portion of the absorbent material, followed by distribution and temperature equilibration, after which the reagent layer is pressed flat and the reaction thus initiated is optically evaluated by reflective photometric methods.

Rapid diagnostic test device and method for its use,
in particular for quantitative determinations

The test strips thus far available on the market for semi-quantitative determinations are generally provided for urine, sometimes also for stool, but seldom for serum, plasma, blood, or similar liquids. These test strips can be brought to reaction simply by dipping into the test solution, since any chromatographic effects that occur are meaningless for a qualitative or semi-quantitative determination. As a rule, however, the determination of various parameters in serum, plasma, or whole blood is meaningful only if a quantitative evaluation of the reaction can be performed at the same time. This is especially true for enzyme determinations as well as for most substrate determinations. Reflective photometric determination methods in particular have gained acceptance for the evaluation of test strip reactions.

When the sample is placed directly on the reaction region of a test strip, chromatographic effects arise which result in a non-uniform distribution of the reagents. This risk must be countered by special measures during the impregnation and in the buildup of the reaction region, resulting in increased costs. In addition, whole blood cannot be readily used since the red blood cells overcolor all other color reactions in the reaction region. For this reason, the serum or plasma must first be extracted in a laborious and time-consuming procedure before the rapid diagnostic test device is used for analysis of blood components. Alternatively, it is known to cover the reaction region with a semi-permeable membrane, or to embed the reagents in a semi-permeable film, from which the red blood cells can be wiped or rinsed off; however, this creates additional difficulties. Furthermore, the reaction proceeds immediately after the sample is applied. In particular for enzyme reactions, however, a measurement is not meaningful until a constant temperature is established. This temperature adjustment can require so much time that a significant portion of the available measurement range is used up.

For this reason, a device which allows the application of the sample to be temporally separated from the wetting of the reaction regions is desirable so that the temperature of the applied sample

can first be equilibrated. At the same time, the device should prevent a chromatographic effect on the reagents used, and allow the use of whole blood. U.S. Patents 3,915,647, 3,917,453, 3,933,594, and 3,936,357 describe devices which allow the application of the sample to be temporally separated from the wetting of the reaction region. The devices are based on the fact that the sample is placed on an absorbent material between two plates or sheets, at least one of which is transparent. The required reagents for the reaction are applied to these plates or sheets by suitable means. The two plates or sheets are kept separate from the absorbent material upon which the sample is applied by using spacing elements, different in each patent, having a complicated design. To apply the sample to the absorbent material, the two plates or sheets are held apart from one another and a metered quantity of the sample is placed on the absorbent material. The plates or sheets are then pressed together by suitable means, whereupon the sample wets the reaction layer and the reaction starts. Using the transparent plate or sheet, the reaction can be evaluated or measured using suitable instruments. Any excess sample is drawn off by suitable means. In addition to the temporal separation of the sample application from the wetting of the reaction region, chromatographic effects are essentially eliminated using these devices, since the reagent layer makes flat contact with the absorbent material wetted by the sample.

A disadvantage of these devices, however, is that the plates must be held apart from one another when the sample is applied, as made apparent from the description of the referenced patents. Serum and plasma, the sample materials used here, are frequently contaminated with hepatitis viruses or other pathogens and thus represent a potential source of dangerous infections. For this reason, utmost avoidance of direct contact with such materials is one of the hygienic measures adopted as a matter of course in the medical field. The known devices are not suited for this purpose. A further disadvantage is that whole blood obviously cannot be used. A third disadvantage is that the necessary spacing elements have such a complicated design that a simple manufacture appropriate for a test strip is clearly not possible, since products based on the referenced patents have thus far not appeared on the market.

German Unexamined Patent Application 30 29 579.5 describes a means of extracting plasma or serum from whole blood, based on the fact that suitable glass fiber layers have filtration properties with respect to red blood cells. The Unexamined Patent Application describes horizontal and vertical filtration devices combined with the reagent regions. For vertical

filtration, the test area must subsequently be exposed by tearing off the non-woven glass fiber fabric which is filled with red blood cells. This is disadvantageous for hygienic reasons, since the infectious sample material can easily splash, or the user can be easily contaminated when grasping the material to be torn off. In the other embodiments provided for test strips, the heparinized plasma or serum infiltrates horizontally into the reaction region. This once again results in strong chromatographic effects which can be avoided, if at all, only by laborious and time-consuming measures, since they would otherwise interfere with a quantitative evaluation of the reaction.

It was unexpectedly found that the structure characterized in greater detail in the claims advantageously ensures a temporal separation of sample application and start of reaction, as well as a uniform, flat wetting of the reaction layer without the disadvantages of the prior art.

Specialized structures are illustrated in the figures, without limiting the invention to same.

Figure 1 shows a side view of a device according to the invention. On a substrate 1 an absorbent material 2 is applied which is simultaneously able to transport liquids. Such materials comprise primarily glass fibers, but also plastic grids, various synthetic non-woven fabrics, and paper. The transport properties of many materials are improved by impregnation with detergents. A transparent sheet 3 together with a reagent layer 4 is affixed to one side of substrate 1 in such a way that fastening 5 is situated next to the absorbent material, and the sheet covers reagent layer 4 from above. The fastening material, preferably a sheet coated on both sides with adhesive, should be somewhat thicker than material 2. Reagent substrate 4 may be either a film optionally having multiple layers and made of a swellable or gel-like plastic which is applied to the sheet, or one or more absorbent papers or similar material. At its unattached end the upper sheet extends for a distance over absorbent material 2, but one region 2a of absorbent material 2 remains uncovered.

Alternatively, according to Figure 1a the reagent layer may be affixed directly to absorbent material 2 using a thin strip of double-sided adhesive tape or a thicker adhesive layer. Fastening 5 must be formed over the narrowest area possible, since this area is no longer available for the

reaction. The adhesive must be stable with respect to the test solution to prevent detachment of the reaction region.

If a sample corresponding to the absorption capacity of the absorbent material is placed on region 2a, the sample becomes distributed uniformly over the entire material 2. Surprisingly, in the structure shown here the sample does not cross over into reagent layer 4, even when the sample is situated directly over absorbent material 2. However, a hydrophobized zone 6 can still be applied at the edge of the absorbent material which faces fastening 5. In any case, a uniform distribution of the sample material over absorbent material 2 is obtained without wetting reagent layer 4. The strip can now advantageously be placed in a temperature equilibration device if this is necessary for an enzyme determination, for example. However, if absorbent material 2 has been impregnated with chemical substances, after the sample is applied, reactions can proceed prior to the actual indicator reaction, which, if needed, produces improved results. Thus, the enzyme creatin kinase (CK) must first be activated with SH reagents before it shows a maximum reaction rate. For this reason it is advantageous to impregnate the absorbent material with *N*-acetyl cysteine, for example, which then activates the CK after the sample is applied.

After temperature equilibration or preliminary reactions have been completed, which may be from a few seconds to approximately 5 minutes, upper transparent sheet 3 together with reagent layer 4 situated thereunder, and, optionally, hydrophobic grid 7 (see Figure 5) are pressed flat onto the absorbent substrate. This pressure may be applied manually, or, to achieve the greatest possible uniformity in colors, by machine, such as by rollers or pressure springs, or by passing through an aperture, or by similar means. In each case, reagent layer 4 is uniformly wetted as a result of the flat pressure, thereby avoiding chromatographic effects. This results once again in the development of uniform reaction colors, which by use of the transparent sheet can be evaluated, either visually or using instrumentation, preferably by reflective photometric methods.

Figure 2 shows a side view of another device in which substrate sheet 1 is formed by deep-drawing, for example, to produce a raised supporting surface 1a for affixing the reagent layer.

Figures 3 and 3a show side views of devices according to Figures 1 and 2 in which an additional separating material 2b is applied over region 2a. This layer 2b facilitates application of the

sample, and when made of a suitable material it can have an added filtering and separating effect. Because of the additional absorption capacity of this layer, the volume of sample applied must be increased in comparison to the device according to Figure 1.

Figure 4 shows the side view of a device for which filtration layer or separation layer 2b is applied next to, but in liquid contact with, absorbent layer 2, a hydrophobized zone 6 improving the separating effect of the layer.

Absorbent layer 2 in this case is preferably made of a material which is very absorbent or not suited for filtration or separation, preferably a synthetic or cellulose non-woven fabric, or also a plastic grid.

Figure 5 shows a side view of a device in which absorbent material 2 is in contact with reagent layer 4 via a hydrophobic grid, the reagent layer in turn being covered by a transparent sheet 3. If pressure is exerted on layer 3, and thus indirectly on layer 2, the test liquid crosses over through grid 7 into layer 4, thereby initiating the reaction.

Reagent layer 4 is made of an absorbent or swellable material commonly used for test strips which is impregnated with the required reagents and auxiliary agents. Preferred are thin filter papers or thin films, optionally applied to the underside of sheet 3, which have a gel-like, blush polymeric, or open structure according to Unexamined German Patent Application 29 10 134.6, or are produced according to German Patent 15 98 153. Slight hydrophobization can be advantageous in reducing premature crossover of the test liquid. It is also possible to combine multiple reagent-containing layers.

Absorbent material 2 should be highly absorbent to ensure rapid transport of the test liquid and to ensure stability with respect to the test liquid and the reagents used. A sponge-like structure is advantageous, in that a variable quantity of test liquid results in uniform saturation without forming a supernatant, and also in that when pressed together with reagent layer 4 a sufficient quantity of liquid is delivered to same. Filter paper, non-woven fabrics, and woven fabrics made of natural or artificial fibers, as well as porous gels made from high-molecular substances (cellulose, proteins, etc.), for example, can be used.

A particularly advantageous embodiment results when absorbent material 2 is produced from glass fibers for separating plasma or serum from whole blood according to Unexamined German Patent Application 30 29 579. It is then possible to use whole blood directly as test liquid, so that a plasma or serum extraction on the test strip can be advantageously combined with the possibility of temporal separation of sample application from wetting of the reaction regions.

Substrate sheet 1 is preferably made of an organic plastic (for example, PVC, polyethylene, polyester, modified cellulose, etc. being suitable), but also may be made of waterproof cardboard or other solid substrate. Cover sheet 3 is likewise made from an organic plastic sheet, but in contrast to the substrate sheet must be transparent to allow the discoloration of the reagent layer to be evaluated. Therefore, this layer should also be as thin as possible, but no more than is just sufficient to have adequate rigidity together with the reagent layer.

Several examples are intended to illustrate the application without limiting the invention. Cholesterol is used as the parameter to be detected, although it is understood that the formulations can also be adapted to other test reactions, for example for glucose, uric acid, lactate dehydrogenase, and other important diagnostic parameters for blood, provided that they result only in reactions that can be optically evaluated. Such reactions for many diagnostic parameters are known to those skilled in the art.

Example 1

A reaction mixture comprising

16 g	partially acetylated cellulose
86 g	0.2% methylhydroxypropyl cellulose
1.00 g	wetting agent (Na-dioctylsulfosuccinate)
12 g	polyvinyl propionate dispersion
0.48 g	3,3',5,5'-tetramethylbenzidine
10 g	titanium dioxide
9600 U	cholesterol oxidase
7200 U	cholesterol esterase
1.04×10^4 U	peroxidase
0.01 g	gallic acid

was coated in a thickness of 0.15 mm on a transparent polycarbonate sheet 110 µm thick, and dried. A strip of this sheet 1 cm wide, with the reaction layer underneath according to Figure 1, was then affixed to a plastic strip on which 15 mm of the glass fiber non-woven fabric having a thickness of 1.5 mm and a fiber thickness of approximately 2 µ had previously been applied, so that the free end of the coated sheet extended 6 mm over the non-woven fabric. This material was then cut into test strips 6 mm wide. When 15 µL whole blood was placed on sample application region 2a corresponding to Figure 1, the plasma portion permeated the entire glass fiber non-woven fabric as well as underneath the transparent sheet within 30 to 60 seconds, whereas the red blood cells were retained in region 2a. Pressing the sheet caused the coating compound in reagent layer 4 to come into contact with the emitted plasma and uniformly saturate same. The cholesterol contained in the plasma reacted, giving a blue coloration with an intensity proportional to the amount of cholesterol.

Example 2

An impregnation solution having the following composition was prepared:

Enzyme impregnation solution

0.45 g	KH_2PO_4
1.55 g	$Na_2HPO_4 \times H_2O$
1.5×10^4 U	cholesterol oxidase
1×10^4 U	cholesterol esterase
3×10^5 U	peroxidase
2.0 g	Na-dioctylsulfosuccinate
250 g	bidistilled H_2O

Teabag paper from [ ][1] was impregnated with this solution and carefully dried (enzyme paper). This paper was cut into strips 1 cm wide.

The following impregnation solution was also prepared:

Indicator solution

2.0 g	3,3',5,5'-tetramethylbenzidine
2.0 g	Na-dioctylsulfosuccinate
250 g	acetone

An additional teabag paper was impregnated with this solution and dried (indicator paper). This paper was cut into strips 1 cm wide.

A transparent polycarbonate sheet 1 cm wide and 110 µm thick, together with the indicator paper and the enzyme paper according to Figure 1, was affixed on one side to a plastic strip so that the sheet was on the outside, the indicator paper was in the middle, and the enzyme paper was on the inside. A glass fiber non-woven fabric 15 mm wide according to Example 1 was placed alongside, as described in Example 1. The structure was cut into test strips 6 mm wide. After the application of 15 µL whole blood, plasma was emitted which permeated the entire glass fiber non-woven fabric (30 to 60 seconds). By pressing transparent sheet 3 according to Figure 1, both reaction papers 4 came into flat contact with the emitted plasma and were saturated with same.

This initiated the reaction of the cholesterol in the plasma, resulting in a blue dye with an intensity proportional to the amount of cholesterol.

[1] Translator's note: Company name is omitted in original document.

Reference Numbers

1	Substrate
1a	Support surface
2	Absorbent material
2a	Sample application region
2b	Additional separating material
3	Transparent sheet
4	Reagent layer
5	Fastener
6	Hydrophobized zone
7	Hydrophobized grid